adding a set of oligonucleotide primer pairs to said sample, wherein said set of oligonucleotide primers comprises at least one oligonucleotide primer pair capable of specifically amplifying a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of pathogenic *E. coli*, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains;

subjecting said sample and said set of primer pairs to an amplification process to produce amplified product; and

detecting the presence of amplified product.

22. The method according to claim 21, wherein the set of oligonucleotide primer pairs comprises primer pairs selected from the group consisting of:

a primer pair that hybridizes to a gene encoding heat labile toxin, or a gene encoding heat stabile toxin for amplification of a DNA sequence characteristic for enterotoxigenic *E. coli*;

a primer pair that hybridizes to a gene encoding heat stabile toxin for amplification of a DNA sequence characteristic for enteroaggregative *E. coli*;

a primer pair that hybridizes to the pCVD432 plasmid for amplification of a DNA sequence characteristic for enteroaggregative *E. coli*;

a primer pair that hybridizes to the inv-plasmid for amplification a DNA sequence contained in enteroinvasive *E. coli*;

a primer pair that hybridizes to the EAF plasmid, or the eae gene for amplification of a DNA sequence characteristic for enteropathogenic *E. coli*; and

a primer pair that hybridizes to the genes encoding shiga-like toxin sltI or sltII for amplification of a DNA sequence characteristic for enterohemorrhagic *E. coli*.

23. The method according to claim 22, wherein the oligonucleotide primer pair that hybridizes to the gene encoding heat labile toxin characteristic for enterotoxigenic *E. coli* is

LT-I: $^{5'}$ GCG TTA CTA TCC TCT CTA TGT G $^{3'}$ (SEQ ID NO.: 1) and
LT-2: $^{5'}$ AGT TTT CCA TAC TGA TTG CCG C $^{3'}$ (SEQ ID NO.: 2);

the oligonucleotide primer pair that hybridizes to the gene encoding heat stabile toxin characteristic for enterotoxigenic *E. coli* is

ST-1: $^{5'}$ TCC CTC AGG ATG CTA AAC CAG $^{3'}$ (SEQ ID NO.: 3) and
ST-2a: $^{5'}$ TCG ATT TAT TCA ACA AAG CAA C $^{3'}$ (SEQ ID NO.: 4);

the oligonucleotide primer pair that hybridizes to the gene encoding heat stabile toxin characteristic for enteroaggregative *E. coli* is

EASTI-1: $^{5'}$ AAC TGC TGG GTA TGT GGC TGG $^{3'}$ (SEQ ID NO.: 5) and
EASTI-2: $^{5'}$ TGC TGA CCT GCC TCT TCC ATG $^{3'}$ (SEQ ID NO.: 6) ;

the oligonucleotide primer pair which hybridizes to the pCVD432 plasmid is

EA-1: $^{5'}$ CTG GCG AAA GAC TGT ATC ATT G $^{3'}$ (SEQ ID NO.: 7) and
EA-2: $^{5'}$ TAA TGT ATA GAA ATC CGC TGT T $^{3'}$ (SEQ ID NO.: 8);

the oligonucleotide primer pair which hybridizes to the inv-plasmid is

EI-1: $^{5'}$ TTT CTG GAT GGT ATG GTG AGG $^{3'}$ (SEQ ID NO.: 9) and
EI-2: $^{5'}$ CTT GAA CAT AAG GAA ATA AAC $^{3'}$ (SEQ ID NO.: 10);

the oligonucleotide primer pair which hybridizes to the EAF plasmid is

EP-1: $^{5'}$ CAG GGT AAA AGA AAG ATG ATA AG $^{3'}$ (SEQ ID NO.: 11) and
EP-2: $^{5'}$ AAT ATG GGG ACC ATG TAT TAT C $^{3'}$ (SEQ ID NO.: 12);

the oligonucleotide primer pair which hybridizes to the eae gene is

EPeh-1: $^{5'}$ CCC GCA CCC GGC ACA AGC ATA AG $^{3'}$ (SEQ ID NO.: 13) and
EPeh-2: $^{5'}$ AGT CTC GCC AGT ATT CGC CAC C $^{3'}$ (SEQ ID NO.: 14);

the oligonucleotide primer pair which hybridizes to the gene encoding shiga-like toxin SltI is

SltI-1: $^{5'}$ ATG AAA AAA ACA TTA TTA ATA GC $^{3'}$ (SEQ ID NO.: 15) and

SltI-2 $^{5'}$ TCA CYG AGC TAT TCT GAG TCA AGC $^{3'}$ (SEQ ID NO.: 16); and

the oligonucleotide primer pair which hybridizes to the gene encoding shiga-like toxin SltII is

SltII-1: $^{5'}$ ATG AAG AAG ATR WTT RTD GCR CYT TTA TTY G $^{3'}$ (SEQ ID NO.: 17) and

SltII-2: $^{5'}$ TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC $^{3'}$ (SEQ ID NO.: 18)

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

1 SubE2 D Cont

24. The method according to claim 21, further comprising an oligonucleotide probe capable of hybridizing to a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of pathogenic *E. coli,* said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein said oligonucleotide probe is labeled at the 5'end with a fluorescent dye and at the 3' end with a fluorescent quencher dye and is susceptible to 5'-3' exonuclease degradation by a polymerase, and wherein said amplification process uses a polymerase having 5'-3' exonuclease degradation activity.

25 The method according to claim 24 wherein the labeled oligonucleotide probe is specific for the respective virulence factor/toxin gene to be detected.

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26. The method according to claim 25 wherein

the labeled oligonucleotide probe is specific for the detection of heat labile toxin gene characteristic for enterotoxigenic E. Coli;

the labeled oligonucleotide probe is specific for the detection of heat stabile toxin gene characteristic for enterotoxigenic E. Coli;

the labeled oligonucleotide probe is specific for the detection of heat stabile toxin gene characteristic for enteroaggregative E. Coli;

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the labeled oligonucleotide probe is specific for the detection of pCVD432 plasmid;

the labeled oligonucleotide probe is specific for the detection of the inv-plasmid;

the labeled oligonucleotide probe is specific for the detection of the EAF-plasmid;

the labeled oligonucleotide probe is specific for the detection of the eae gene;

the labeled oligonucleotide probe is specific for the detection of shiga-like toxin SltI gene; and

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the labeled oligonucleotide probe is specific for the detection of shiga-like toxin SltII gene.

27. The method according to claim 26 wherein the labeled oligonucleotide probe for the detection of heat labile toxin gene characteristic for enterotoxigenic *E. coli* is

$^{5'}$ AGC TCC CCA GTC TAT TAC AGA ACT ATG $^{3'}$ (SEQ ID NO.: 19)

the labeled oligonucleotide probe for the detection of heat stabile toxin gene characteristic for enterotoxigenic *E. coli* is

$^{5'}$ ACA TAC GTT ACA GAC ATA ATC AGA ATC AG $^{3'}$ (SEQ ID NO.: 20);

the labeled oligonucleotide probe for the detection of heat stabile toxin gene characteristic for enteroaggregative *E. coli* is

$^{5'}$ ATG AAG GGG CGA AGT TCT GGC TCA ATG TGC $^{3'}$ (SEQ ID NO.: 21);

the labeled oligonucleotide probe for the detection of pCVD432 plasmid is

$^{3'}$ CTC TTT TAA CTT ATG ATA TGT AAT GTC TGG $^{3'}$ (SEQ ID NO.: 22);

the labeled oligonucleotide probe for the detection of the inv-plasmid is

$^{5'}$ CAA AAA CAG AAG AAC CTA TGT CTA CCT $^{3'}$ (SEQ ID NO.: 23)

the labeled oligonucleotide probe for the detection of the EAF-plasmid is

$^{5'}$ CTT GGA GTG ATC GAA CGG GAT CCA AAT $^{3'}$ (SEQ ID NO.: 24);

the labeled oligonucleotide probe for the detection of the eae gene is

$^{5'}$ TAA ACG GGT ATT ATC AAC AGA AAA ATC C $^{3'}$ (SEQ ID NO.: 25);

the labeled oligonucleotide probe for the detection of shiga-like toxin SltI gene is

$^{5'}$ TCG CTG AAT CCC CCT CCA TTA TGA CAG GCA $^{3'}$ (SEQ ID NO.: 26);

the labeled oligonucleotide probe for the detection of shiga-like toxin SltII gene is

$^{5'}$ CAG GTA CTG GAT TTG ATT GTG ACA GTC ATT $^{3'}$ (SEQ ID NO.: 27).

28. The method according to claim 24 wherein the fluorescent reporter dye is 6-carboxy-fluorescein, tetrachloro-6-carboxy-fluorescein, or hexachloro-6-carboxy-fluorescein, and the fluorescent quencher dye is 6-carboxytetramethyl-rhodamine.

29. The method according to claim 21 wherein the amplification process comprises 35 PCR cycles at a MgC12 concentration of 5.2 mmol, an annealing temperature of 55 °C and an extension temperature of 65 °C.

30. A set of oligonucleotide primer pairs useful for polymerase chain reaction (PCR) amplification of DNA of pathogenic enterobacteria allowing detection and differentiation of pathogenic enterobacteria in a sample, wherein each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of the pathogenic *E. coli* strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for amplification of each subgroup at least one oligonucleotide primer pair is included in said set of oligonucleotide primer pairs.

31. The set of primer pairs according to claim 30 comprising

a primer pair that hybridizes to a gene encoding heat labile toxin, or to a gene encoding heat stabile toxin of enterotoxigenic *E. coli*;

a primer pair that hybridizes to a gene encoding heat stabile toxin of enteroaggregative *E. coli*;

a primer pair that hybridizes to the pCVD432 plasmid of enteroaggregative *E. coli*;

a primer pair that hybridizes to the inv-plasmid of enteroinvasive *E. coli*;

a primer pair that hybridizes to the EAF plasmid, or the eae gene of enteropathogenic *E. coli*; and

a primer pair that hybridizes to the gene encoding shiga-like toxin sltI or sltII of enterohemorrhagic *E. coli*.

32. The set of primer pairs according to claim 31 wherein

the primer pair which hybridizes to the gene encoding heat labile toxin of enterotoxigenic *E. coli* is

LT-1: 5' GCG TTA CTA TCC TCT CTA TGT G 3' (SEQ ID NO.: 1) and
LT-2: 5' AGT TTT CCA TAC TGA TTG CCG C 3' (SEQ ID NO.: 2);

the primer pair which hybridizes to the gene encoding heat stabile toxin of enterotoxigenic *E. coli* is

ST-1: 5' TCC CTC AGG ATG CTA AAC CAG 3' (SEQ ID NO.: 3) and
ST-2a: 5' TCG ATT TAT TCA ACA AAG CAA C 3' (SEQ ID NO.: 4);

the primer pair which hybridizes to the gene encoding heat stabile toxin of enteroaggregative *E. coli* is

EASTI-1: 5' AAC TGC TGG GTA TGT GGC TGG 3' (SEQ ID NO.: 5) and

EASTI-2: $^{5'}$ TGC TGA CCT GCC TCT TCC ATG $^{3'}$ (SEQ ID NO.: 6);

the primer pair which hybridizes to the pCVD432 plasmid is

EA-1: $^{5'}$ CTG GCG AAA GAC TGT ATC ATT G $^{3'}$ (SEQ ID NO.: 7) and
EA-2: $^{5'}$ TAA TGT ATA GAA ATC CGC TGT T $^{3'}$ (SEQ ID NO.: 8);

the primer pair which hybridizes to the inv-plasmid is

EI-1: $^{5'}$ TTT CTG GAT GGT ATG GTG AGG $^{3'}$ (SEQ ID NO.: 9) and
EI-2: $^{5'}$ CTT GAA CAT AAG GAA ATA AAC $^{3'}$ (SEQ ID NO.: 10)

the primer pair which hybridizes to the EAF plasmid is

EP-1: $^{5'}$ CAG GGT AAA AGA AAG ATG ATA AG $^{3'}$ (SEQ ID NO.: 11) and
EP-2: $^{5'}$ AAT ATG GGG ACC ATG TAT TAT C $^{3'}$ (SEQ ID NO.: 12);

the primer pair which hybridizes to the eae gene is

EPeh-1: $^{5'}$ CCC GGA CCC GGC ACA AGC ATA AG $^{3'}$ (SEQ ID NO.: 13) and
EPeh-2: $^{5'}$ AGT CTC GCC AGT ATT CGC CAC C $^{3'}$ (SEQ ID NO.: 14);

the primer pair which hybridizes to the shiga-like toxin sltI gene is

SltI-1: $^{5'}$ ATG AAA AAA ACA TTA TTA ATA GC $^{3'}$ (SEQ ID NO.: 15) and
SltI-2: $^{5'}$ TCA CYG AGC TAT TCT GAG TCA AGC $^{3'}$ (SEQ ID NO.: 16); and

the primer pair which hybridizes to the shiga-like toxin sltII is

SltII-1: $^{5'}$ ATG AAG AAG ATR WTT RTD GCR GYT TTA TTY G $^{3'}$ (SEQ ID NO.: 17) and

SltII-2: $^{5'}$ TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC $^{3'}$ (SEQ ID NO.: 18)

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

33. A set of labeled oligonucleotide probes useful for detection and differentiation of pathogenic enterobacteria in a sample by Real Time–PCR, each probe specifically binding a sequence of a virulence factor/toxin genes characteristic of one of the subgroups of pathogenic *E. coli* strains comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for detection and differentiation of each subgroup at least one probe is included in the set of oligonucleotide probes.

34. The set of probes according to claim 33 comprising

a labeled oligonucleotide probe specific for the detection of heat labile toxin gene characteristic for enterotoxigenic E. Coli;

a labeled oligonucleotide probe specific for the detection of heat stabile toxin gene characteristic for enterotoxigenic E. Coli;

a labeled oligonucleotide probe specific for the detection of heat stabile toxin gene characteristic for enteroaggregative E. Coli;

a labeled oligonucleotide probe specific for the detection of pCVD432 plasmid;

a labeled oligonucleotide probe specific for the detection of the inv-plasmid;

a labeled oligonucleotide probe specific for the detection of the EAF-plasmid;

a labeled oligonucleotide probe specific for the detection of the eae gene;

a labeled oligonucleotide probe specific for the detection of shiga-like toxin SltI gene; and

a labeled oligonucleotide probe specific for the detection of shiga-like toxin SltII gene.

35. The set of probes according to claim 34 wherein

the labeled oligonucleotide probe for the detection of heat labile toxin gene characteristic for enterotoxigenic *E. coli* is

$^{5'}$ AGC TCC CCA GTC TAT TAC AGA ACT ATG $^{3'}$ (SEQ ID NO.: 19);

the labeled oligonucleotide probe for the detection of heat stabile toxin gene characteristic for enterotoxigenic *E. coli* is

$^{5'}$ ACA TAC GTT ACA GAC ATA ATC AGA ATC AG $^{3'}$ (SEQ ID NO.: 20);

the labeled oligonucleotide probe for the detection of heat stabile toxin gene characteristic for enteroaggregative *E. coli* is

$^{5'}$ ATG AAG GGG CGA AGT TCT GGC TCA ATG TGC $^{3'}$ (SEQ ID NO.: 21);

the labeled oligonucleotide probe for the detection of pCVD432 plasmid is

$^{5'}$ CTC TTT TAA CTT ATG ATA TGT AAT GTC TGG $^{3'}$ (SEQ ID NO.: 22);

the labeled oligonucleotide probe for the detection of the inv-plasmid is

$^{5'}$ CAA AAA CAG AAG AAC CTA TGT CTA CCT $^{3'}$ (SEQ ID NO.: 23)

the labeled oligonucleotide probe for the detection of the EAF-plasmid is

$^{5'}$ CTT GGA GTG ATC GAA CGG GAT CCA AAT $^{3'}$ (SEQ ID NO.: 24)

the labeled oligonucleotide probe for the detection of the eae gene is

$^{5'}$ TAA ACG GGT ATT ATC AAC AGA AAA ATC C $^{3'}$ (SEQ ID NO.: 25)

the labeled oligonucleotide probe for the detection of shiga-like toxin SltI gene is

$^{5'}$ TCG CTG AAT CCC CCT CCA TTA TGA CAG GCA $^{3'}$ (SEQ ID NO.: 26);

the labeled oligonucleotide probe for the detection of shiga-like toxin SltII gene is

$^{5'}$ CAG GTA CTG GAT TTG ATT GTG ACA GTC ATT $^{3'}$ (SEQ ID NO.: 27).

36. A set of oligonucleotide primer pairs and a set of oligonucleotide primer probes useful for diagnosing an enterobacteria infection in samples derived from a living animal body including a human, by Real time PCR method, wherein said set of oligonucleotide primer pairs allows detection and differentiation of pathogenic enterobacteria in a sample, wherein each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of the pathogenic *E. coli* strains said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for amplification of each subgroup at least one oligonucleotide primer pair is included in said set of oligonucleotide probes and a set of oligonucleotide probes, wherein said set of oligonucleotide probes detect virulence factor/toxin genes characteristic of one of the

subgroups of pathogenic *E. coli* strains , said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains by real time PCR

37. The method of claim 21, wherein said method is used to diagnose an enterobacteria infection in a sample derived from a living animal body.

38. The method of claim 27, wherein said sample is derived from a human.

39. The method of claim 21, wherein said method is used to detect enterobacteria contamination of a consumable.

40. The method of claim 29, wherein said consumable is selected from the group consisting of meat, milk or vegetable.

REMARKS

Claims 1-20 are pending in this application and have been canceled without prejudice or disclaimer. Claims 21–40 are newly added. Support for the newly added claims may be found throughout the specification. For example, support for claim 21 may be found at page 4, lines 13-16 and page 5, lines 15-20; for claims 22 and 31 at page 6, lines 8-30, page 7, lines 1-6 and page 11, lines 8-26; for claims 24 and 33 at page 7, lines 10-35, page 8, lines 1-30, page 12, lines 1-30 and page 13, lines 1-23; for claim 25 at page 4, lines 14-16; for claims 26, 27, 34 and 35 at page 9, lines 15-30, page 10, lines 15-30, page 14, lines 5-31 and page 15, lines 1-8; for claim 28 at the bridging paragraph on pages 10-11; for claim 29 at page 11, lines 3-5, for claim 30 at page 11, lines 8-26, page 14, lines 6-31 and page 15, lines 1-2; for claim 36 at page 11, lines 8-26, page 14, lines 6-31 and page 15, lines 1-2 and for claims 37-40 at the bridging paragraph at pages 19-20 and page 15, lines 6-8. Thus, no new matter has been added . Reconsideration of the application in view of the following remarks is respectfully requested.